STUDIES IN THE METABOLISM OF NORMAL AND REGENERATING TISSUE OF THE EARTHWORM.

PART I. FACTORS AFFECTING THE ENDOGENOUS OXYGEN CONSUMPTION OF NORMAL AND REGENERATING MUSCLE TISSUE.

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(Eight Text-figures.)

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Introduction.

The purpose of this investigation was to establish certain information related to the techniques used for the examination of respiratory mechanisms, functioning throughout the processes of tissue repair and reorganization. Experimental work on the respiratory activity of annelid tissue has been in most cases to develop the concept of Metabolic Gradient elaborated by Child and his school.

Respiratory activity as a function of segmental level has been investigated in several of the Polychaetes and Oligochaetes. Hyman and Galegher (1921) obtained a U-shaped curve describing the antero-posterior gradient for oxygen consumption (Qo_2), in two forms of *Nereis* and one species of *Lumbriculus*, the estimation of oxygen being carried out according to the Winkler method. Shearer (1924) demonstrated that the anterior Qo_2 was approximately twice that of the posterior, using both small pieces of worm and acetone powders. Perkins (1929) confirmed the result obtained by Hyman and Galegher, in that he obtained a similar gradient curve for Qo_2 in *Lumbricus* and *Allolobophora*. In an effort to ascertain whether a relation existed between "growth" metabolism and this gradient he estimated the total iodine equivalence, –SH, and total S, but found no correspondence with the observed Qo_2 data. Okada (1929) and Kawaguti (1934) obtained a U-shaped curve for both Qo_2 and Qco_2 in an Oligochaete Branchiura.

Maloeuf (1935), using very small pieces of worm in order to eliminate motor activity, concluded that no significant difference occurred in the Qo_2 at different levels of the earthworm, and that the effect obtained by other workers was due to motor activity in the pieces examined.

In general the work done on the respiratory activity among the Annelida appears to demonstrate that a gradient in Qo_2 exists. Little work has been carried out either utilizing manometric techniques or from the viewpoint of the relative response of metabolic systems to the growth and organization requirements imposed upon the organism following injury. Hyman (1932), using the Winkler technique, examined the Qo_2 of *Nereis virens* after injury and in the case of posterior tissue obtained a depression in Qo_2 relative to the normal tissue and concluded that posterior tissue following injury exhibits a subnormal Qo_2. With reference to the development of this project it was necessary to consider the possible sources of variation which may arise both within the tissue of the organism and in the methods of investigation when comparisons are made between experiments differing in design. The following report has been confined to the determination of such conditions as appear optimal for the survival and metabolism of the tissue during experimental treatment, and to the investigation of the endogenous oxygen consumption of early regeneration tissue.

Material.

The organism concerned was a species of earthworm, belonging to the genus *Allolobophora*. The population from which samples were taken was obtained from garden soil at Mosman, Sydney. The culture was maintained in glass troughs filled

with damp earth. The worms were fed on crumbed bread and periodically the earth was replaced.

The tissue used was the muscular layer, consisting essentially of circular and longitudinal muscle bounded externally by a thin cuticular layer.

Methods and Results.

(i) *Selection of experimental lots. Specimens for experimentation were selected in the following manner*: The worms when first obtained were segregated into four size classes, A^+, A, B, and C, defined within certain limits of the total length, and the diameter of the post-clitellum region. These limits are indicated in Table 1.

TABLE 1.

Class.	Total Length (cm.).	Diam. (cm.).
A^+	>14	>0·4
A	11–14	0·3–0·4
B	7–10	0·2–0·25
C	5– 7	0·1–0·2

These dimensions obtained from anaesthetized, relaxed specimens form classes into which worms may be placed at sight with little difficulty.

Material for experiments was then selected without bias from the respective classes consisting mainly of 50 worms, which in turn was selected from a population of 200 or 250 per group, there being one or more of the latter groups per size class, depending upon the number of worms in the entire culture.

(ii) *Preparation of tissue.*—The portion of the worm required was cut off, split longitudinally, and the viscera, including the nerve cords, carefully scraped away from the muscle. The muscle strip was then washed rapidly in two changes of distilled water, dried carefully on filter paper and placed on a glass slide over ice. When sufficient tissue had been prepared the strips were transferred to a clean dissecting board, cut into squares approximately equal in area, thoroughly mixed and transferred as equivalent portions to glass slides of known weight. The slide plus tissue was weighed and the latter transferred to the Warburg vessels, the medium in which was ice-cold. Thus the mixed tissue mass from which that required for each experimental unit was obtained, was composed of tissue from several worms, the number dependent on experimental requirements. A variation may arise because of the selection of different numbers in certain experiments. In view of this the relationship between the number selected from a lot and the consistency of the result obtained was examined within the limits of the work concerned. Table 2 indicates that the variation between lot 1, 2, 3, of Set I

TABLE 2.

Set.	Lot.	No./Lot.	No. Selected.	Wt. of Tissue.	ul. O_2/60 min.
I	1	50	10	100 mg.	14·1
	2	50	20	100 mg.	13·8
	3	50	50	100 mg.	14·2
II	1	50	10	200 mg.	30·2
	2	50	20	200 mg.	30·8
	3	50	50	200 mg.	31·8
III	1	50	20	100 mg.	14·6
	2	50	20	100 mg.	14·1
	3	50	20	100 mg.	14·3

and Set II is no greater than that between three lots of the same number, Set III. Throughout the work the procedure followed has been a selection of 20 worms per lot of 50.

(iii) *Measurement of Qo_2.*—The oxygen consumption was measured by the standard Warburg technique (Warburg, Dixon, Umbreit), the tissue squares being suspended in 3 c.c. of an aqueous salt solution. The CO_2 was absorbed by 20% KOH and paper. The manometers were shaken at approximately 90 oscillations per minute in a bath at a temperature of 27°C.

(iv) *Medium.*—The following media for the suspension of the tissue were investigated:

Medium.	NaCl (gm.).	KCl (gm.).	$MgSO_4 7H_2O$.	$CaCl_2$ (gm.).	$(PO_4.)$	H_2O (c.c.).
Ringer	0·864	0·023	0·038	—	—	100
Ringer–PO_4	0·864	0·023	0·038	—	—	100
Amphibian Ringer–PO_4	0·650	0·014	—	0·012	—	100
Krebs–Hensleit	0·900	0·046	0·037	0·038	—	100
Krebs–Hensleit–PO_4	0·900	0·046	0·037	0·038	—	100
M/100 PO_4—buffer distilled water	0·5 ml. of M/15 PO_4 buffer per 3 ml. H_2O					

The media containing phosphate are made up by adding 0·5 ml. of M/15 PO_4-buffer to the medium concerned in the respirometer flask. The pH of the media was adjusted to 7·5 by addition of N/10 NaOH where required.

The investigation is recorded in Table 3 and shows little difference between the three Ringer solutions and the PO_4-buffer solution, whereas both the Krebs-Hensleit solutions and the distilled water alone, gave low values for the Qo_2. Throughout the following work Amphibian-Ringer-PO_4 was the medium selected, this being similar in freezing point depression to earthworm blood, which has a value −0·45 to −0·51 (Heilbrunn).

TABLE 3.

Medium.	pH.	Wet Wt. Tissue.	Temp. °C.	ul O_2/hr.
Ringer	7·5	200 mg.	35° C.	44·7
Ringer–PO_4	,,	,,	,,	43·1
Amphibian–Ringer–PO_4	,,	,,	,,	46·5
Krebs–Hensleit	,,	,,	,,	30·6
Krebs–Hensleit–PO_4	,,	,,	,,	27·6
PO_4 buffer (M/100)	,,	,,	,,	42·2
Distilled water	,,	,,	,,	28·4

(v) *Tissue mass.—Fluid volume variation*: The amount of tissue per flask was selected with two ends in view—namely, to yield an easily measurable oxygen uptake and to conserve tissue. In general approximately 100 mgs. wet weight was considered sufficient. Table 4 indicates the Qo_2 for different weights of tissue. In all cases the volume of the medium was 3·0 mil. per flask, consequently by varying the amount of tissue the tissue/volume ratio is changed. The effect of an increase in the value of this factor is apparent from both Tables 4 and 5, where the departure from a linear relationship between tissue weight and Qo_2 is marked.

TABLE 4.
Temp. 37° C. pH 7·5.

Wet Wt. Tissue.	30 Min.	60 Min.	90 Min.
50 mg.	3·7 ul.	10·9 ul.	16·5 ul.
100 mg.	13·4 ,,	24·4 ,,	34·5 ,,
200 mg.	34·0 ,,	56·1 ,,	88·5 ,,
400 mg.	74·4 ,,	134·5 ,,	199·0 ,,

Table 5 expresses the Qo_2 over a period of 120 minutes for three tissue/volume ratios, of 0·18, 0·08 and 0·06, relative to three positions along the worm, viz., (1) the terminal posterior 10 segments, (2) the 11th–20th, and (3) 21st–40th.

TABLE 5.
Temp. 27° C. pH. 7·5.

Wt. tissue (wet) mg.	550			250			180		
Tissue/ vol. ratio	0·183 ≒ 0·2			0·083 ≒ 0·1			0·060 ≒ 0·05		
Time (min.) ..	0–10 (A_1)	10–20 (A_2)	21–40 (A_3)	0–10 (B_1)	11–20 (B_2)	21–40 (B_3)	0–10 (C_1)	11–20 (C_2)	21–40 (C_3)
	ul.	ul.	ul.	ul.	ul.	ul.	ul.	ul.	ul.
35 min. ..	80·5	74·6	55·5	37·4	28·0	17·1	17·4	17·0	16·2
60 min. ..	128·5	119·5	92·0	64·2	46·5	42·0	33·0	34·2	30·8
120 min. ...	238·5	216·3	168·0	114·0	84·1	73·0	57·3	56·3	53·5

In order to minimize the variation between experiments where the tissue/volume ratio differs the extent of this error should be ascertained and the experimental results adjusted before comparison is made. Fig. 1 shows the Qo_2 of Table 5 plotted as a function of time, the Qo_2 expressed as ul of O_2/100 mg. wet weight tissue. It can be readily seen that as the ratio increases beyond 0·05 the error introduced by reducing results to ul/100 mg. tissue becomes considerable.

(vi) *Limiting thickness of tissue.*—The importance of this source of error has been stressed by those workers concerned with tissue slice experiments; an additional factor may be introduced by this tissue, namely, its increase in thickness due to a contraction effect during the experiment. Both the limiting thickness and the increase due to contraction have been investigated. Squares of tissue, prepared as described, were dried carefully, weighed, and the surface area measured, from which values an approximate measure of thickness was obtained, assuming the tissue density to be unity. Table 6 gives the result for the average thickness of mixed slices from worms belonging to classes A^+, A, and B.

TABLE 6.

Size Class.	No. Samples.	Mean Thickness.
A	100	0·5 mm.
A	,,	0·35 mm.
B	,,	0·22 mm.

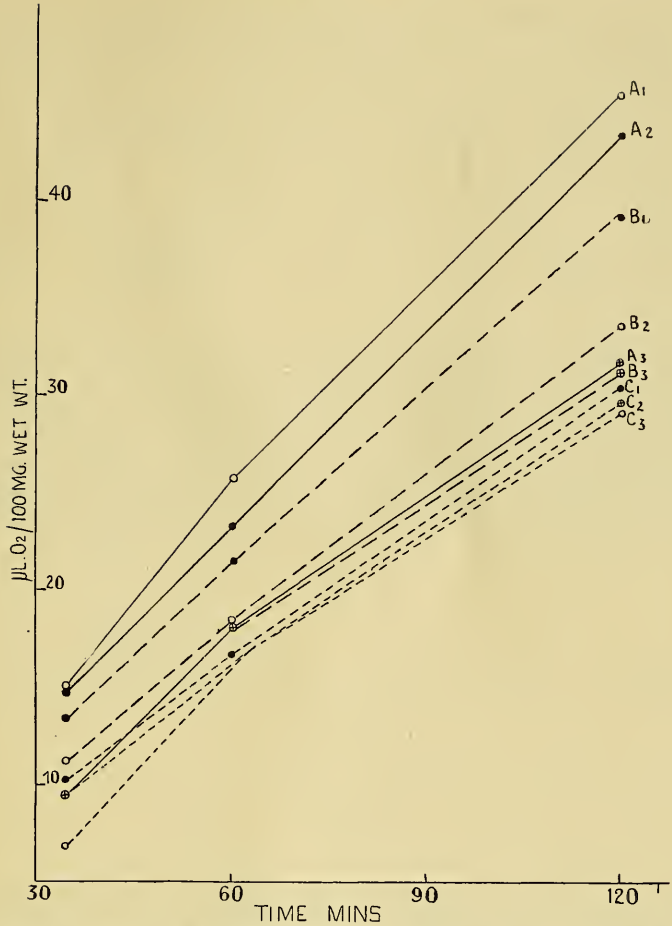


Fig. 1.—Relation between Qo_2 expressed as ul of O_2/100 mg. wet wt. of tissue, and time.

Table 7 shows that a small increase in thickness occurs after the tissues have been shaken in a flask for 30 minutes. This increase may be due to a contraction effect produced by the medium.

TABLE 7.

Size Class.	Sample No.	Av. Initial Thickness.	Av. Final Thickness.	Percentage Increase.
A	100	0·31 mm.	0·34 mm.	12

These results were then examined according to the formula devised by Warburg, which is claimed to yield a reliable estimate of the limiting thickness for O_2 consumption in a given tissue. The limiting thickness d′ is given by:

$$d' = \sqrt{8\ Co\ \frac{D}{A}}$$

where Co = Concentration of O_2 outside tissue.
D = Diffusion const. for O_2 in ml. at N.T.P.
Krogh's value for tissue at 38°C. = $1{\cdot}4 \times 10^{-5}$
A = O_2 consumption of tissue per unit volume and time.

Table 8 shows the values of d′ calculated from data obtained from Tables 4 and 6.

TABLE 8.

Class.	Mass Tissue (gm.).	Vol. Tissue (c.c.).	Time Min.	Mls. of O_2.	d′.
A	0·100	0·1	60	$24·4 \times 10^{-3}$	0·7 mm.
A	0·200	0·2	90	$88·5 \times 10^{-3}$	0·6 mm.
A	0·050	0·05	60	$10·9 \times 10^{-3}$	0·7 mm.

If 0·6 mm. is taken to be the limiting value then the tissue used throughout should not be subject to diffusion limitations. Tissue from worms of class A^+ was considered unacceptable since when the 12% contraction effect is added it tends to approach the limiting value when the possible error of this factor is also considered.

(vii) *Oxygen Pressure.*—The effect of an increase in oxygen concentration was examined in order to provide an additional check on the question of limiting thickness. Gas mixtures containing 20%, 70%, and 100% oxygen were made up from commercial cylinders of air and pure oxygen. The results are indicated in Table 9.

TABLE 9.

Size Class.	Experiment.	ul O_2/100 mg. wet wt./hr.		
		20% O_2.	70% O_2.	100% O_2.
B	I	16·3	16·9	12·6
B	II	17·1	—	15·7
B	III	15·9	15·7	13·6

This additional evidence suggests that no limitation due to diffusion of oxygen resulted from the tissue thickness used. The depression in Qo_2 observed in pure oxygen does not concern this report and will be covered in greater detail in further work. It was considered that any difference between the 20% and 70% values was not significant and that the gas phase could safely be air.

(viii) *Area of tissue squares.*—The question of the relationship between the oxygen consumed and area of tissue square was examined. Class B worms were used and squares prepared of three surface area values. Table 10 indicates that a slight inverse relation may exist between these factors. This appears to be to some extent a function of area *per se* as indicated by a diminution in the effect at a higher oxygen tension; however, an additional factor due to increase in tissue damage where the smaller squares are concerned may also be important.

TABLE 10.

Size Class.	Mean Area (mm.²).	ul O_2/Mg. total N/hour.		
		20% O_2.	70% O_2.	100% O_2.
B	18·3	11·6	17·5	12·2
	9·7	—	14·7	14·5
	4·3	17·8	18·1	17·9

In view of the difficulties involved in estimating an effect due to tissue damage in any quantitative manner it was the practice throughout to perform all experiments at a constant "tissue area" value. The value selected was that approximating 4·3 mm.^{2} and the error involved in preparation was of the order $\pm$ 1 mm.^{2}. Thus if comparative experiments are run with similar tissue mass and similar area of tissue squares the possibility of errors due to a function of area and tissue damage are minimized.

(ix) *Size of worm in relation to* Qo_2.—Worms of the three size classes, A, B, and C, were taken and two tissue regions prepared from each specimen. (i) Tissue from the 15 posterior terminal segments and (ii) tissue from the 15th–40th posterior segments. Fig. 2 indicates a variation between the groups concerned; however, it is considered that, although the difference between the A-worms and the two smaller may be significant, that between B- and C-worms is not significant, being due to experimental error. Throughout this work worms of size class B have been used unless otherwise stated.

(x) *Segmental level in relation to* Qo_2.—A variation in Qo_2 was found to exist between the terminal posterior segments and those anterior to them. It was considered necessary to investigate the extent of this variation in relation to segmental level, with the aim to select some portion of the posterior tissue which showed a relatively constant Qo_2. Posterior tissue was prepared from a lot of 50 worms and divided into the following categories:

(*a*) Terminal 10 segments.
(*b*) 11th to 20th segments.
(*c*) 21st to 35th segments.
(*d*) 35th to 55th segments.
(*e*) 55th to 70th segments.

The results are indicated in Table 11 and Fig. 3.

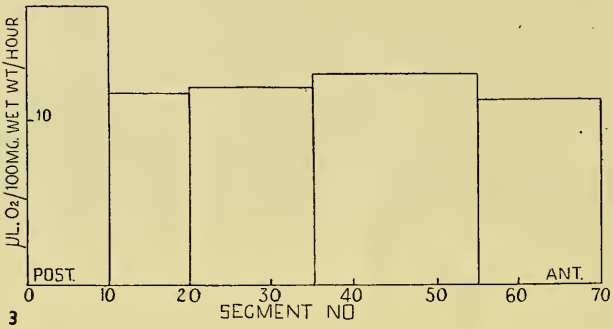
TABLE 11.

Exp.	Qo_2 as ul O_2/100 mg. wet weight.				
	0–10 (a)	11–20 (b)	21–35 (c)	35–55 (d)	55–70 (e)
I	16·7	12·0	—	—	11·6
II	17·5	11·8	12·1	13·0	10·0
III	17·4	12·1	11·9	12·4	12·6
Mean ..	17·2	11·9	12·0	12·7	11·4

A higher Qo_2 value is observed in the terminal group of segments than in those anterior to them up to the level of the 70th segment, and the respiratory activity appears to show little variation between the 10th and 70th segment. This result is also indicated in Fig. 2, where the terminal group of 15 segments exhibits a higher Qo_2 than the group 15th–40th. In order to ensure a comparatively stable rate of oxygen consumption tissue for examination was taken from the 20th to the 40th segmental level unless otherwise stated.

(xi) *pH effect.*—The Qo_2 was investigated over a range of pH values from 5·8 to 8·6. The medium used was Amphibian Ringer made up in M/100 PO_4-buffer at the pH required, N/10 NaOH was added to obtain the 8·6 value. The pH was checked at the conclusion of each experiment with a glass electrode. No alteration was found to have occurred. Fig. 4 shows the Qo_2 obtained plotted as a function of pH, and indicates the presence of a plateau between 7·4 and 8·2 at 27°C., whereas Fig. 5 shows the optimum shifted to a lower value as the temperature increased. These results indicated that the pH should be maintained at 7·4 to 7·5 throughout the work.

(xii) *Temperature effect.*—Experiments were carried out over a range from 16°C. to 40°C., the duration of an experiment being 90 minutes. At 40°C. no drop in the respiratory rate had occurred. Fig. 6 summarizes these observations. The effect on Qo_2 over the above range in relation to previous environmental temperatures was examined. Worms were kept at room temperature (20°C.–23°C.), at 25°C. and at 28°C. No variation in results was noticed, so that any error arising from the fact that the organisms were not maintained in an environment of constant temperature was negligible; however, in the experiments to follow dealing with the regenerating tissue the worm population and the experimental lots were maintained at 27°C.



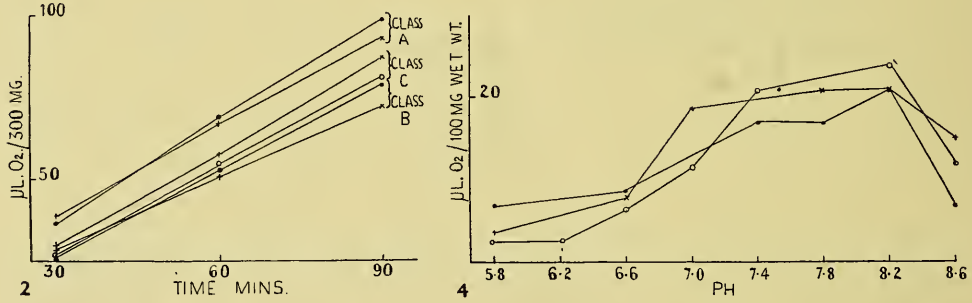


Fig. 2.—Qo_2 plotted against time for three size classes, A, B, C, and two tissue regions. O — terminal 15 segments + — 15th to 40th post. segments.

Fig. 3.—Histogram prepared from Table 11 relating Qo_2 and position of tissue (as segment No.) on the worm.

Fig. 4.—Qo_2 as ul. O_2/100 mg. wet wt. of tissue as a function of pH.

(xiii) *Expression of results.*—Results are expressed as ul of O_2 consumed by 100 mg. wet weight of tissue and in some cases the Qo_2 is related to total nitrogen. In consequence of this a number of estimations were carried out in which the relationship between Wet Weight, Dry Weight and Total N was ascertained. The relation between Wet Weight and Dry Weight may be expressed by the regression equation $y = 5 + 5{\cdot}1x$ where x and y are dry and wet weight respectively; and that between Dry Weight and Total Nitrogen by the equation $y = -0{\cdot}2 + 12{\cdot}5x$ where x represents Total Nitrogen.

(xiv) *Estimation of Total Nitrogen.*—Total Nitrogen was estimated in the following manner: At the conclusion of an experiment the KOH-paper was removed from the respirometer flask and any excess KOH neutralized by addition of a few drops of sulphuric acid (approximately 50%). The flask contents were then washed into a one-inch diameter boiling tube. The tubes were then placed upright in an oven and the contents evaporated to dryness; 0·5 ml. of 50% sulphuric acid, together with several drops of hydrogen peroxide, were then added and the contents digested over a small flame for 30 minutes. The digest was then washed into a 100 ml. volumetric flask made up to 100 ml. and set aside for analysis.

The analysis carried out was a typical Nesslerization followed by colorimetric comparison with a standard.

Endogenous Oxygen Consumption Following Injury.

(i) *Qo_2 following injury at the 30th segmental level.*—Approximately 300 worms were selected; 150 were injured by cutting off the posterior 30 segments; 150 remained uninjured. Each lot was returned to a separate container filled with similar moist earth and maintained at 27°C. At periods of 24 hours, 72 hours, and 168 hours a sample of 30 specimens was taken at random from each lot and tissue prepared. The tissue of the regenerating portion consisted of the terminal 5 segments as less was difficult to prepare rapidly. Consequently as the worms regenerated a higher percentage of the tissue taken consisted of regenerate. At 24 hours about 10% wet weight was regenerate, at 48 hours about 30%, at 72 hours 50% and at 168 hours approximately 70–80%. These figures were obtained by previously examining regenerating worms and weighing the apparently new tissue formed, hence may involve considerable error as in the early stage much may be mucus or damaged tissue rather than new tissue and in the later stages a low estimate may occur following difficulty in distinguishing the regenerating tissue. However these estimates are given mainly as an indication of the relative amounts of tissue in the sample.

Two tissue portions were taken from the normal uninjured worms: (1) the terminal 10 segments and (2) the tissue from the 30th to 40th levels. The normal tissue was included so as to indicate possible change in Qo_2 following the injury and to enable comparison to be made between the two normal levels of oxygen uptake and that accompanying regenerative activity. The results obtained are given in Table 12, the Qo_2 expressed as ul of O_2 per mg. total nitrogen, and in Fig. 7, where Qo_2 is represented as a function of time.

Table 12.

Exp.	Time (min.).	24 Hrs.			48 Hrs.			72 Hrs.			168 Hrs.		
		R	N_1	N_2	R	N_1	N_2	R	N_1	N_2	R	N_1	N_2
I	15	1·1	1·3	2·1	0·9	0·9	—	0·8	1·0	1·9	2·3	1·0	2·1
	30	3·2	3·0	5·3	3·3	—	6·7	4·2	3·1	4·6	6·3	3·9	5·7
	60	7·0	7·4	14·0	8·2	8·5	12·9	10·1	7·1	13·1	11·4	8·3	12·6
II	15	1·0	—	—	0·8	1·0	2·2	—	—	—	2·4	0·8	2·0
	30	4·1	—	—	3·9	4·5	8·1	4·5	4·4	8·6	7·8	5·0	8·7
	60	7·5	—	—	9·2	7·6	13·7	11·2	10·0	14·3	13·2	8·2	14·8

R = Tissue from regenerating portion of injured earthworm.
N_1 = Tissue from 30th-40th segs. normal worm.
N_2 = Tissue from 0-10th segs. normal worm.

(ii) *Endogenous Qo_2 and effect of previous injury.*—With further work in view it was felt necessary to consider the possibility of variation arising as a function of previous, recent injury. The experiment was arranged so that two periods, 48 hours and 72 hours following injury, were studied. Each period contained the following groups:

(*a*) Worms regenerating at the 30th segmental level for first time.

(*b*) Worms regenerating at the 30th segmental level following a previous injury at that level 48 hours before section.

(*c*) Worms regenerating at the 30th segmental level following two previous injuries at that level 48 hours apart and 48 hours prior to section.

By the 30th segment is understood the original segmental level of the first injury.

The results are indicated in Table 13, and are referred to 100 mg. wet weight of tissue.

TABLE 13.

Time (min.).	48 Hrs. After Injury.			72 Hrs. After Injury.		
	No. of Previous Injuries.			No. of Previous Injuries.		
	0	1	2	0	1	2
15	1·1	1·0	—	1·1	—	1·4
30	5·4	3·2	4·1	5·9	5·9	5·7
60	12·8	11·7	11·9	14·1	13·4	14·3

(iii) *Endogenous Qo_2 in tissue adjacent to injury.*—Worms injured 72 hours previous to the experiment were used. Three portions of tissue were taken from the injured worms:

(*a*) Terminal regenerating portions to approximately the 5th segment.
(*b*) Adjacent tissue from 5th to 10th segment.
(*c*) Tissue from between 40th and 50th segments.

The results are expressed as ul of O_2 per 100 mg. wet weight and are tabulated in Table 14.

TABLE 14.

Worm Tissue.	Segments.	15 Min.	30 Min.	60 Min.
Injured ..	0– 5	1·2	5·9	14·2
	6–10	1·6	3·2	9·5
	30–40	2·2	4·3	10·0
Uninjured ..	0–10	1·7	3·5	12·3
	30–40	2·3	—	9·7

Fig. 8 indicates the increase in Qo_2 occurring in both the terminal segments of the normal worm and in the regenerating portion of the injured, compared with the lower value for tissue adjacent to the injury which exhibits a similar activity to the 30th–40th level on the normal worm.

Discussion of Results.

An attempt has been made to establish experimental procedure for the examination of this particular tissue in order to facilitate further work on this project. The experiments reported are rather to indicate possible sources of variation and the extent of the error involved when comparisons are to be made, than to present a detailed examination of the factors concerned *per se*.

The apparent gradual increase in the Qo_2 of the regenerating portion from the relatively low value typical of the 40th segmental level to the higher value exhibited by the normal terminal segments may be due either to a gradual increase in the metabolic rate of the tissue with time or to an increase in the percentage of tissue characterized by a higher Qo_2, in the sample. If a large proportion of the tissue obtained shows a low Qo_2 value relative to that exhibited by the actual regenerate then any increase due to regenerating tissue would be moderated if only a small proportion of the latter tissue was present. Table 14 and Fig. 8 suggest that the latter explanation

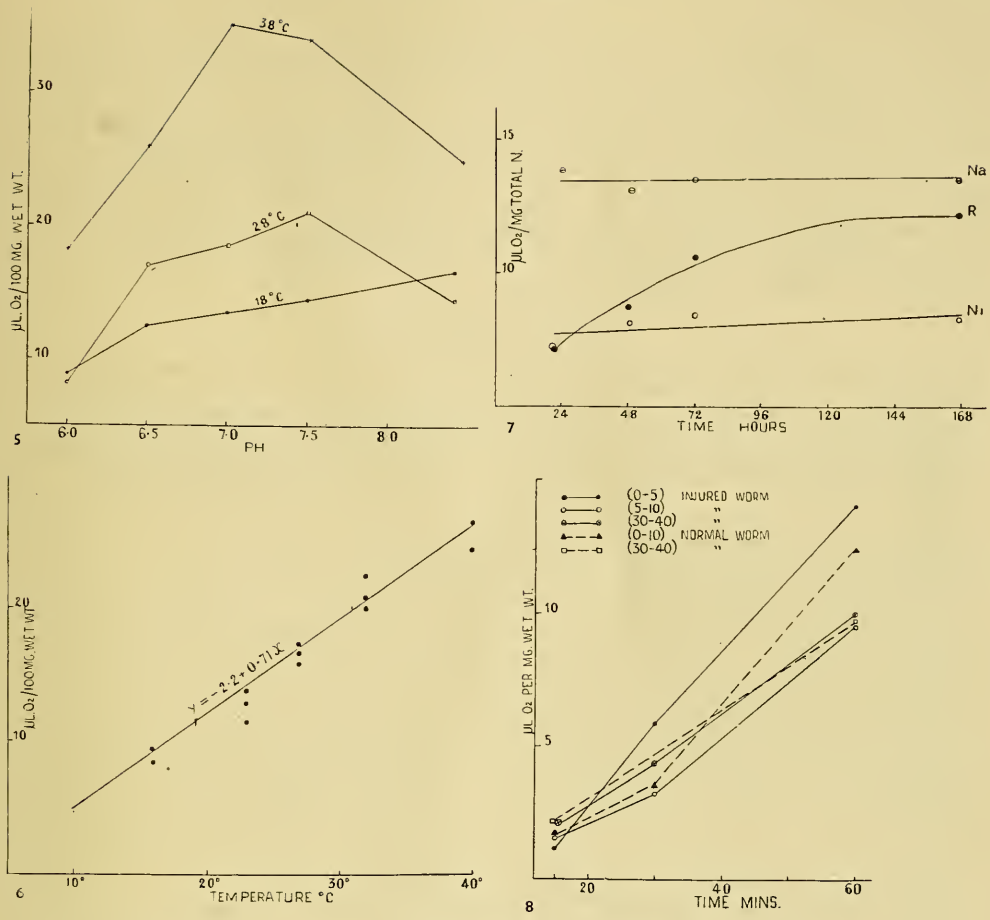


Fig. 5.—Qo_2 plotted as a function of pH at temperatures 18°C., 28°C., 38°C.

Fig. 6.—Qo_2 plotted as a function of temperature over a range from 16°C. to 40°C.

Fig. 7.—Showing the relationship between the respiratory activity of uninjured tissue at two levels. N_1 — level of 30th-40th seg., N_2 — terminal post. tissue and regenerating tissue at 30th segmental level. R, over a period of 168 hours.

Fig. 8.—Respiratory activity of uninjured tissue, regenerating tissue and tissue adjacent to injury.

is possible and that tissue at the 40th segmental level is transformed from one of a relatively low Qo_2 to one characteristic of terminal growing tissue. The question then to be considered is the rate at which this transformation takes place. Taking into account the approximate proportion of regenerate in the sample it would appear that little difference occurs during the first 30 hours but that after this period the apparent transformation time is increased by the depressing effect of uninjured tissue present in the sample. When it is considered that the regenerating tissue is formed rapidly in response to injury and that organization and growth are superimposed upon the initial cellular mass, it is reasonable to expect an increase in metabolic activity and, provided that the metabolic pathways are predominantly aerobic, an increase in Qo_2 should occur. The question of the effect of previous injury is one which is being considered in more detail, especially in relation to food storage and starvation. Table 13 was included to show that in well fed worms recent injury was not an important source of variation within the limits of the experiment described.

The increase in oxygen consumption appears to be related to a large extent to an increase in total dehydrogenase and in particular to succinic dehydrogenase; however, this work is as yet incomplete and will be reported later.

Summary of Results.

(i) The endogenous oxygen uptake exhibited by posterior muscle tissue in the earthworm *Allolobophora* sp. has been investigated.

(ii) Possible sources of variation due to experimental technique have been examined and an indication given of the optimal experimental conditions for this tissue.

(iii) The terminal posterior segments have been shown to maintain a higher Qo_2 than those anterior to them and that the Qo_2 of regenerating tissue at a level of low value increases towards the higher value as regeneration proceeds.

(iv) The effect of previous recent injury was found to be slight and of no great significance, relative to the work concerned.

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